Attorney's Docket No. 035510/296072 PATENT

RESPONSE UNDER 37 C.F.R. 1.116 - EXPEDITED PROCEDURE - EXAMINING GROUP 1653

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	Guarino et al.	Confirmation No.:	4333
Appl. No.:	10/670,771	Group Art Unit:	1653
Filed:	September 26, 2003	Examiner:	Marsha M. Tsay
For:	PEPTIDES FOR ENHANCED CELL ATTACHMENT AND GROWTH		

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RULE 37 C.F.R. § 1.131 AFFIDAVIT
of
Richard D. Guarino, Bryce N. Chaney, Andrea Liebmann-Vinson, Perry D. Haaland, and Mohammad A. Heidaran

We, Richard D. Guarino, Bryce N. Chaney, Andrea Liebmann-Vinson, Perry D. Haaland, and Mohammad A. Heidaran, do hereby declare and say as follows:

1. We have each read and understand the patent application of Guarino *et al.*, Application No. 10/670,771 entitled PEPTIDES FOR ENHANCED CELL ATTACHMENT AND GROWTH.

2. We are each inventors of the subject matter that is currently claimed in the above-referenced application.

3. As set forth in the accompanying Invention Disclosure Record (IDR) entitled "Peptides for attachment and maintenance of function of Primary Hepatocytes," subject matter claimed in pending claims 65, 66, 69, 70-78, 80, and 82-85 was conceived of and reduced to practice in the U.S. prior to August 28, 2003 (the publication date of U.S. Patent Application Publication No. 20030162289). Specifically, the completed IDR was submitted prior to August 28, 2003 (dates in the IDR have been redacted, but are all earlier than August 28, 2003); the inventors resided in the U.S. at the time of completion of the subject matter claimed in pending claims 65, 66, 69, 70-78, 80, and 82-85 (see signature blocks on pages 12 and 13); and Figures 1-4 establish the reduction to practice of said subject matter at least by the submission date of the IDR.

4. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Richard D. Guarino — 06/07/06 Date

Bryce N. Chaney — 06/07/06 Date

Andrea Liebmann-Vinson — 6/9/06 Date

Perry D. Haaland — 06/08/06 Date

Mohammad A. Heidaran — Date

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Richard D. Guarino — Date

Bryce N. Chaney — Date

Andrea Liebmann-Vinson — Date

Perry D. Haaland — Date

Mohammad A. Heidaran — 6/7/06 Date

BD

BECTON DICKINSON AND COMPANY
INVENTION DISCLOSURE RECORD

To: Chief Patent and Licensing Counsel Becton, Dickinson and Company 1 Becton Drive Franklin Lakes, NJ 07417	**Date Received:** **Patent Dept. File No.:** P-6099 **Attorney/Agent Assigned:** JDW
Originating Division: BD Technologies **Relates to:** *(select one WWBusiness from pull-down list below)* Corporate Research	**Date Submitted:** **Presumptive Inventors:** Richard D. Guarino, Andrea Liebmann-Vinson, Bryce Nelson Chaney, Perry D. Haaland and Mohammad A. Heidaran
Title: Peptides for attachment and maintainance of function of Primary Hepatocytes	
1. Date of Completion of the Inventive Concept:	**5. Date of First.....** **Publication or Expected Publication:**
2. Date and Location of Earliest Written Description:	**Nature of Publication:** **Sale or Expected Sale:**
3. Date of First Experimental Demonstration of the Invention: **Done by:** NB 02-8283-53 pp 17-20 **Witnesses:**	**In What Countries?** **Showing to Others Outside BD**
4. Date First Shown or Described to Others at BD:	**6. Related Agreements, Licenses or Contracts:**

7. **DETAILED DESCRIPTION OF THE INVENTION:** Describe in detail ***what the invention is***, ***how it works*** and what ***advantages/improvements*** it provides as compared to similar compositions, methods or machines and ***what prior art problems are solved*** by the invention. In addition to describing what you believe to be ***the best embodiment and (if one exists) the commercial embodiment***, include a description of ***all operative variations and alternatives*** in reaction components, concentrations, methods of use, methods of manufacture, mechanical parts, etc. If possible, describe the ***underlying technical principle*** which produces the advantageous result. Include similar descriptions for any ***related aspects of the invention*** which are necessary to make and use it (e.g., methods of making and using a novel compound or composition, methods of using a novel machine). Consider and indicate which of these ***related aspects may also be patentable*** (e.g., if a chemical process, are the reactants or products also novel? If a new chemical composition, are the methods of making it and/or the methods of using it also novel? If a novel machine, is the product it produces also novel in some way?) For the invention and its various related aspects, describe any ***unexpected results*** which were observed. Include or attach ***drawings***, if appropriate, or if they are helpful for understanding the invention (e.g., chemical structure, flow charts, engineering drawings, schematics). All such ***additional pages should be separately witnessed and dated***. Copies of ***laboratory notebooks are not necessary at this time***, but may be cited and should be readily available for future reference.

A. What the Invention Is and How It Works

Using the BioTherapy peptide screen derived from and earlier Media Optimization project (media optimization technology. The peptides were chosen for follow-up designs based on biological activity for either secretion and/or adhesion.

Immobilized Peptide Hits from peptide screen: immobilized peptides following design and designation of contents of peptides in wells are found in protocol "Peptides Immobilized – 40 petides 60 wells" on CATS Bioinformatics data base using MPM.

RPH 12 is the strongest experiment for adhesion, cells very loose in RPH14

In RPH12 peptide screen, the following wells contained live cells after 5 days: See Figures 3 and 4 on attached figures (Although all wells with cells on day 5 are good for attachment, wells with * also were especially of note for attachment on Day 1)

done as run 6/7/1

Well B02: Peptide Group F6, F3

Well C02: Peptide Group F5, F8

* Well C05: Peptide Group F5, F9

*Well C06: Peptide Group F4 only

Well C07: Peptide Group F2, F5

Well D06: Peptide Group F5, F6

* Well D11: Peptide Group F1, F4 (note F1 contains an RGD control, well know in patents+lit)

* Well E02: Peptide Group F5 only

* Well E08: Peptide Group F4, F10

* Well F02: Peptide Group F5, F1(note F1 contains an RGD control, well know in patents+lit)

* Well F04: Peptide Group F5, F10

Well F11: Peptide Group F3, F5

* Well G06: Peptide Group F4, F5

Well G07: Peptide Group F4, F9

Well G11: Peptide Group F5, F7

Note well B07, B11 good hit for cyp but images show large clumps at end of day 5: Maybe attachment not as important for these peptides? Loosely adherent? May be good for soluble peptide experiments?

CYP signal divided up into strong and intermediate groups:

Strong CYP Signal both RPH12 and RPH14 experiments: Wells that gave good 7-ethoxy resorufin signal= 1A1/1A2 CYP activity after 5 days, repeated twice RPH12 and RPH14

Well B07: Peptide Group F4,F6 note cell not adhering well here, in clumps but strong 7ER CYP 1A1 signal

Well B11: Peptide Group F4, F8, note cell not adhering well here, in clumps but strong 7ER CYP 1A1 signal

Well C02: Peptide Group F5, F8

Well C06: Peptide Group F4 only

Well C07: Peptide Group F2, F5

Well D11: Peptide Group F1, F4 (note F1 contains an RGD control, well know in patents+lit)

Well E08: Peptide Group F4, F10

Well G07: Peptide Group F4, F9

Intermediate CYP Signal both experiments, consistently above baseline but not as strong as others

Well B02: Peptide Group F6, F3

Well C05: Peptide Group F5, F9

Well C09: Peptide Group F2,F4: note cell not adhering in well here, in clumps but consistent signal above baseline

Well D04: Peptide Group F4,F7: note cell not adhering in well here, in clumps but consistent signal above baseline

Well D06: Peptide Group F5, F6

Well E04: Peptide Group F10 alone: note cell not adhering in well here, in clumps but consistent signal above baseline

(Continued)

Well F04: Peptide Group F5, F10

Well G11: Peptide Group F5, F7

Well F11: Peptide Group F3, F5

Well G06: Peptide Group F4, F5

Peptide families found consistently in hits above: Wells consistently with functional liver cells over time contain: Group 4 and Group 5:

Group F4: well C06 is Group F4 only, had good cyp activity by itself

AFKIF

AFAFK

FIFAK

KKLVY

Group F5: did not do as well by itself as with combinations of other peptide families

AKKMV

FAKFI

KMILY

KSYYY

AFKIF and KMILY were hits for adhesion from single peptide screen cited below, no functional data on those screens, only live cells after 1 and 8 days.

Note in IDR P-5277, peptide KKLNY (seq ID NO 42) is listed as a hit for secretion enhancing activities: this differs by only one amino acid from F4 KKLVY above, the 2nd to last amino acid.

Note in IDR P-5277, 3 peptides from above are cited as hits for adhesion: AFKIF (group 4), FIFAK (group 4), FAKFI (group 5): these are listed with sequence ID number 9,3, 11 respectively in section 0017 of P-5277

Other Peptide Families Cited: Note, I have not screened IDR P5705 for presence of these peptides, this still need to be done

F1 (note F1 contains an RGD control, well know in patents+lit)

AAIKK

AKIFF

GRGDS (here is the RGD control)

IKYYY

F2

EFIKK

FFFIK

FIKLM

KFAFI

F3

EEEKV

EFIKY

FIKFG

GHK

F6

GPVVY

KKKK

NTVYY

QVVAK

F8

AFIPV

AIKKK

FKFIG

KKMYY

F9

AADMQ

AFFKI

AKKKT

FKLVA

F10

DIKPV

FFHPY

KLLMV

VFPFK

Relevant notebook references

First attachment date NB 02-8283-53 pp 17-20 individual peptide screen, NO FUNCTIONAL data on this experiment, only attachment. Note not witnessed until 05/03 because I did not know the identity of the well hits until much later: because of this, these results become follow-up of RPH12 peptide screen

RPH 12, NB 03-8283-22 RPH Primary rat Hep prep 12 peptide screen with first functional data, cyp activity and albumin secretion

p 3 begin experiment; Day 1 pp 4-6; Day 6 pp 6-8; discussion results, key observations, 8-10; 1st demonstration of definitive cyp results p 7; cyp graphs p 11,12,25; Day 1+5 graphs of live cells using nuclear stain p 34; albumin assay data and graph, pp 35,36: MPM output of peptide content of each well: p 37-39, determination of which peptide families are active for attachment and CYP activity: p 43; email detailing results of peptide screen to group: p 44

images of cells on peptide plates: pp 27-33:

RPH 14, NB 03-8283-22 p 47:

B. Advantages and Improvements Over the Prior Art

Novel peptides for maintenance of liver function, out of a labara of screened peptides, only these showed activity for adhesion and maintained of liver function, could replace RDG based peptides for adhesion and maintenance of liver function

1) Enables defined mammalian cell culture conditions by eliminating the need for serum (=undefined mixture of proteins!) in the culture. The cell attachment process is controlled by

the covalently immobilized peptide versus a non-specific and randomly and arbitrarily adsorbed serum protein layer on the cell culture substrate.

2) Use of covalently attached peptides, is preferred over using conventional passive (adsorbed) peptide coatings because the peptide is restricted to the cell culture substrate and cannot be solubilized into the liquid phase (media). Solubilized peptide from conventional passive coatings can interact with attachment sites on a cell while the cell is suspended in the liquid, taking up this attachment site and can thus lead to a reduction of cell attachment to the passive coating. This process is eliminated when using covalently attached peptides

3) Cells grown on immobilized peptides; can more readily be applied in cellular therapies because the need for undefined growth reagents, e.g. serum is eliminated. This leads to a) a significantly reduced risk of cell culture contamination by using biohazardous agents and b) eliminates the need to use animal products that could cause immunogenic rejection of cells grown in this environment and used for cell therapy (implantation).

Eliminate the use of undefined animal products such as Matrigel, which also has variable composition from lot to lot. The lack of animal products is a critical to the progress in the area of cellular therapy.

C. Solutions to Prior Art Problems and How They Are Accomplished

ClickNtype

D. Best Embodiment and (if one exists) Commercial Embodiment

Peptides for maintenance of liver function in any unique cellular environment,

1) traditional cell culture applications using human primary liver cells or other cells derived from liver

2) special uses of cell culture where serum free environments are critical such as determination of function or cellular process using human primary liver cells.

3) environments for drug discovery and drug screening using human primary liver cells, including toxicity and proliferation assays, or assays that determine potential drug targets

4) determination of the effect of drugs or new chemical entities on the biology stem cells: for example experiments exploring effects of drugs on cellular function (intercellular signaling cascades), potential cancer induction

5) use of this environment for applications in cellular therapies and tissue engineering, including devices (such as liver assist devices), scaffolds and other biotherapeutic applications that utilize human primary liver cells in conjunction with any of the unique environments described in this IDR: could be used as a therapeutic

6) Use of same environments for human epithelial stem cells or liver derived cell lines. This is significant because human epithelial stem cells could be a potential therapeutic: even from the same person? Or from a donor liver

7) Use of peptides as a therapeutic

This list is not intended to be a complete list: broadly, any use of epithelial stem cells in cell culture for a wide variety of applications

E. Operative Variations and Alternatives

In addition to immobilized peptides, may be active in solution either with single peptides or in combination with others. Peptides could be attached to other surfaces or other bioactive molecules to increase viability of primary liver cells. Could be used for in vivo liver cellular therapy, in vivo cell culture to maintain liver cell viability, for drug discovery applications, especially where function is important.

F. Underlying Technical Principle(s)

Peptides were covalently immobilized on a non-fouling hyaluronic acid surface using attachment chemistry previously described in other liver related IDR's (see below)

The underlying technical principals for the use of covalent attachment chemistry, i.e. HA-peptide plates allows are described here:

Typically in cell and tissue cultures, a media containing serum is placed on a synthetic cell culture substrate such as tissue culture grade polystyrene (PS). Under these conditions, instantaneous non-specific protein adsorption occurs on the PS surface generating a protein layer consisting of many different proteins in a spectrum of conformational states ranging from almost native to highly denatured [1]. Within minutes, cells arrive and start to interrogate this poorly organized interface via cellular integrins, proteoglycans and selectins [2-7]. Interactions with this randomly adsorbed protein layer leads to arbitrary biological responses in a variety of processes including attachment, spreading, proliferation, migration and differentiation [2-7]. In contrast, normal biological reactions occur via specific and organized ligand-receptor interactions and this in turn triggers highly organized signaling processes [7-11].

There is thus a need for highly defined cell culture surfaces that mimic the in vivo specificity of biological events to more effectively support cell biology during in vitro cell and tissue culture.

Our approach involves two steps: (1) the elimination of non-specific protein adsorption by masking surfaces with an inert coating that will prevent non-specific cell attachment (according to process disclosed in BDT IDR P-5416); (2) introduction of specific cell adhesion sites onto the inert coating by incorporating cell adhesion molecules, in this case Collagen 6, onto such inert surfaces (according to process disclosed in BDT IDR "Method of functionalizing polystyrene plastic by the use of activated glycosaminoglycans").

Specifically, we employ the non-adhesive properties of hyaluronic acid (HA), to create inert coatings on PS surfaces. In order to create inert coatings on PS a method described by Morra et al. [12] can be used. Polystyrene culture dishes, 96-well plates or slides were exposed to an oxidizing radiofrequency plasma treatment, followed by exposure to a polyethyleneimine (PEI) solution to introduce reactive amine groups on the surface. A carbodiimide/succinimide supported condensation reaction of a primary amine with a carboxylic acid was used to form a covalent bond between the PEI coating and the polysaccharide. Alternatively, amine groups introduced on polystyrene surfaces during the Primaria™ plasma treatment or on a poly-lysine coating (instead of PEI) can be used (P5416).

Next, conventional bioconjugation techniques including sodium periodate oxidation and reductive amination, are used to covalently couple Collagen 6 to the inert HA (IDR "Method of functionalizing polystyrene plastic by the use of activated glycosaminoglycans"). Any non-covalently attached extracellular matrix protein is removed by a salt-acid wash followed by rigorous rinsing with water.

This process creates a well-defined surface consisting of covalently immobilized extracellular matrix protein on a non-fouling (=eliminating non-specific cell attachment) background provided by HA.

Alternatively, alginate (also know as alginic acid) could be used as the non-adhesive background and ECM proteins can be immobilized onto this surface using the same chemistry as described above for HA. Also, other commonly known non-adhesive surfaces, such as polyHEMA or PEG (also known as PEO) could be used in combination with a variety of chemistries to couple ECM proteins that are described in the literature (Jeff Hubbell).

1. Norde, W., Adsorption of Proteins from Solution at the Solid-Liquid Interface. Advances in Colloid and Interface Science, 1986. 25: p. 267-340.

2. Elbert, D.L. and J.A. Hubbell, Surface Treatments of Polymers for Biocompatibility. Annu. Rev. Mater. Sci., 1996. 26: p. 365-394.

3. Grinnell, F., Cellular Adhesiveness and Extracellular Substrata. Int. Rev. Cytol., 1978. 53: p. 65-144.

4. Horbett, T.A. and M.B. Schway, Correlations between mouse 3T3 cell spreading and serum fibronectin adsorption on glass and hydroxyethylmethacrylate-ethylmethacrylate copolymers. Journal of Bimedical Materials Research, 1988. 22: p. 763-793.

5. Horbett, T.A., The role of adsorbed proteins in animal cell adhesion. Colloids and Surfaces B: Biointerfaces, 1994. 2: p. 225-240.

6. Drumheller, P.D., C.B. Herbert, and J.A. Hubbell, Bioactive Peptides and Surface Design, in Interfacial Phenomena and Bioproducts, J.L. Brash and P.W. Wojciechowski, Editors. 1996, Marcel Dekker: New York. p. 273-310.

7. Horbett, T.A. and L.A. Klumb, Cell Culturing: Surface Aspects and Considerations, in Interfacial Phenomena and Bioproducts, J.L. Brash and P.W. Wojciechowski, Editors. 1996, Marcel Dekker, Inc.: New York. p. 351-445.

8. Webb, K., V. Hlady, and P.A. Tresco, Relationships among cell attachment, spreading, cytoskeletal organization, and migration rate for anchorage-dependent cells on model surfaces. Journal Biomedical Materials Research, 2000. 49: p. 362-368.

9. Horbett, T.A., Protein Adsorption on Biomaterials, in Biomaterials: Interfacial Phenomena and Applications, S.L. Cooper and N.A. Peppas, Editors. 1982, American Chemical Society: Washington, DC. p. 233-244.

10. Hubbell, J.A., Biomaterials in Tissue Engineering. Biotechnology, 1995. 13: p. 565-576.

11. Golumbfskie, A.J., V.S. Pande, and A.K. Chakraborty, Simulation of biomimetic recognition between polymers and surfaces. PNAS, 1999. 96(21): p. 11707-11712.

12. Morra, M., et al., Process for the coating of objects with hyaluronic acid, derivatives thereof, and semisynthetic polymers, US 6,129,956, 2000.

G. Description of Related Aspects of the Invention (are they also potentially patentable?)

It may possible that you may get similar effects using peptides that are passively added instead of covalently attached. Also, there may be biological activity of peptides in solution that bind receptors either specifically or non-specifically that cause intercellular changes that help maintain liver function. This needs to be further explored by further experimentation and optimization.

H. Unexpected Results

Novel peptides for maintenance of liver function, out of a large library of screened peptides, only these showed activity for adhesion and maintained of liver function

I. Description of the Attached Drawings

Rat Primary Hepatocytes were bought commercially by Xenotech (Hepatoch, Inc) and were isolated by the vendor using standard collagenase digestion methods. Cells were delivered to BD Technologies within 3 hours of the preparation. Cells were seeded at an initial density of 20,000 cell/well in fully supplemented BD Hepatostim commercial media with 2% fetal bovine serum onto plates containing different combinations of covalently attached peptides. Plates were placed in CO2 37°C and were allowed to incubate overnight (Day 1) or for 5 Days. Media was changed every other day by removing half the volume of media from the plates and adding the same volume of fresh media. Peptide families: each family contains 4 peptides as indicated below). Either one family or 2 families of peptides were added across the plates and covalently coupled to a non-fouling surface.

Materials and methods for Assays: at days 1 and 5, duplicate plates were taken for assays as described below. Three assays were run on the same plates: CYP 1A1/2A activity assay, nuclear stains for cell counting and albumin elisas for albumin secretion.

CYP1A1 activity assay and cell enumeration with nuclear stains . All media was transferred to separate plates and media samples were frozen at –20 C until elisa assays for albumin secretion could be performed (see below). 5 uM 7-ethoxy resorufin + 80 uM dicumerol was added to all wells with cells and read every minutes for 30 min on a BMG Polarstar at excitation= 540 nm and emission=590 nm to detect CYP1A1/1A2 activity. 7- ethoxycoumarin was aspirated and nuclear stain with 10 uM Hoechst 33334 stain (Molecular probes, cat # 3570) and 2 mM ethidium homodimer-1 (Molecular probes, Dead stain cat # L-3324) in BDT base media was added to each well. Plates were incubated for 30 min at room temperature and fluorescence images were captured an HT Imager (Discovery-1, Universal Imaging Corporation) at excitation of 405 nm and emission of 480 nm for the Hoechst stain and excitation of 535 nm and emission of 750 nm for the ethidium homodimer stain (10X magnification, 4 sites per well). Cell numbers were determined using UIC Metamorph™ analysis software. Number of live cells was determined by subtracting total cells by dead cells (Hoechst stain-dead stain). Data are presented as the average number of live cells with three replicate plates and error bars represent standard deviation. Peptides-HA experiments were performed 2 separate liver preparations.

Albumin elisa assays: To measure albumin secretion, Probind Assay plates (Falcon 353915) were coated with 2 ug/ml Sheep IGG Anti-rat albumin antibodies, (unconjugated, Cappel cat # 55729) in a bicarbonate buffer (pH=9.6) and allowed to incubate overnight at 4C. Antibody plates were washed 3 X with PBS Tween 20 and blocked with 1% gelatin (Type B, 75 bloom, Sigma cat #G6650) in PBS Tween 20 for 30 min at 37C. Blocking solution was rinsed off 3X with PBS Tween 20 and 1:400 diluted albumin/media samples from peptides screens were added along with a rat albumin (Purified

Rat Albumin, Cappel cat #55952) standard curve (serially diluted 1:2 from 50 ng/ml to 1.56 ng/ml with duplicates for each dilution). Plate were incubated 1 hr at 37C. Plates were washed 3X with PBS Tween 20 and conjugated antibody (peroxidase conjugated Sheep IGG Anti-rat albumin antibodies, Cappel #55776 diluted 1:500 from 36.6 mg/ml) in PBS Tween 20 was added to all wells in antibody plate and incubated at 37C for 1 hr, then washed 3X with PBS Tween 20. 0.25 mg/ml of ABTS substrate for peroxidase (Sigma #A9941) was added to a citrate substrate buffer (pH 5.0) with 0.01% hydrogen peroxide and then added to antibody plates for color development for 40 min at room temp in the dark. The peroxidase reaction was stopped with 0.32 % sodium fluoride solution and absobance was read at 405 nm using a BMG Optima plate reader.

Figure 1A: CYP 1A1/1A2 activity using 7-ethoxycoumarin of rat primary hepatocytes after 5 days in culture on various combinations of covalently attached peptides. Data shows that specific peptide families allow maintenance of CYP activity. The activity is comparable or better than cells placed on standard tissue culture polystyrene.

Figure 1B Same as figure A, except the total CYP fluorescence was lower then most hits in Figure A. Signal is consistently higher then baseline fluorescence, either HA alone or drug only values.

Figure 2A Albumin secretion of peptide hits that maintained CYP activity. Data shows that albumin secretion is maintained in wells and that albumin levels are comparable to control wells of tissue culture polystyrene

Figure 2B: Same as 2A only for the peptide hits with intermediate CYP activity.

Figure 3 Attachment of rat primary liver cells after 18 hour in media containing 2% fetal bovine serum. Data show that certain combinations of covalently attached peptide combinations enhance the attachment of human primary hepatocytes compared to hyaluronic acid only and that the number of attached cells was similar to unmodified polystyrene controls.

Figure 4 Survival of rat primary liver cells after 5 days using nuclear staining. Data show that certain combinations of covalently attached peptide combinations enhance the survival of human primary hepatocytes compared to hyaluronic acid and that the number of attached cells was similar to unmodified polystyrene controls.

8. **RELATED ART:** List the citations of any ***published materials*** which you believe are relevant to evaluating the patentability of the invention. Relevant published materials include published patents and patent applications (domestic and foreign), journal articles, published product information, vendor publications, books, editorials, etc. Also describe any relevant related ***research projects at BD*** and give citations for any relevant related ***BD patent applications or patents*** of which you are aware (by Patent and Licensing Dept. file number or U.S. Serial No.). Please ***attach copies of the cited publications***. It is not necessary to provide copies of BD patent materials.

A. Published Materials

N/A

B. Related Work at BD

P-5769 Col VI environments for liver and non liver cells

P-5839 Environments for Hep G2 human hepatoma cell line

P-5841 environments for RESC Rat liver epithelial cells

P-5994 environments for Human liver cells

P-5277 Peptides promoting cell adherence, growth and secretion

C. Related BD Patents and Patent Applications

P-5277 Peptides promoting cell adherence, growth and secretion, see notebook 03-8283-22 p. 43: overlapping peptides that are cited in this patent already in P5277: AFKIF, FIFAK from F4 and FAKFI from group F5 are also listed as hits for adhesion and/or growth promoting activities in section 0017 of P-5277 "and combinations thereof". Also, KMILY from F5 was also a hit in the single peptide hit

P-5769 Col VI environments for liver and non liver cells

P-5839 environments for Hep G2 human hepatoma cell line

P-5841 environments for RESC Rat liver epithelial cells

P-5994 environments for Human liver cells

First Presumptive Inventor:

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WITNESSES - This document should be witnessed by people who ***understand the invention*** and the technology to which it relates, but who are ***not directly involved*** in it.

Explained to and understood by me:

Signature _____ Date _____

Signature _____ Date _____

Figures for IDR "Attachment and maintenance of function of primary hepatocytes" by RD Guarino et al: F values refer to peptides families

Figure 1A: CYP 1A1/1A2 activity on cells attached to immobilized peptides: strong CYP hits

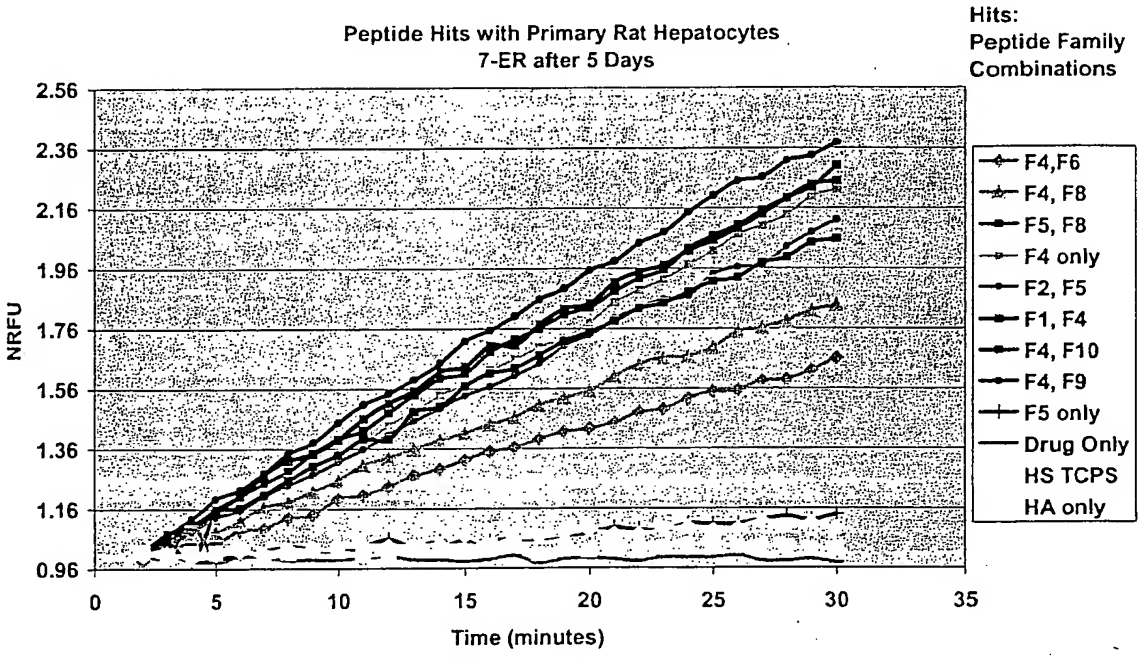
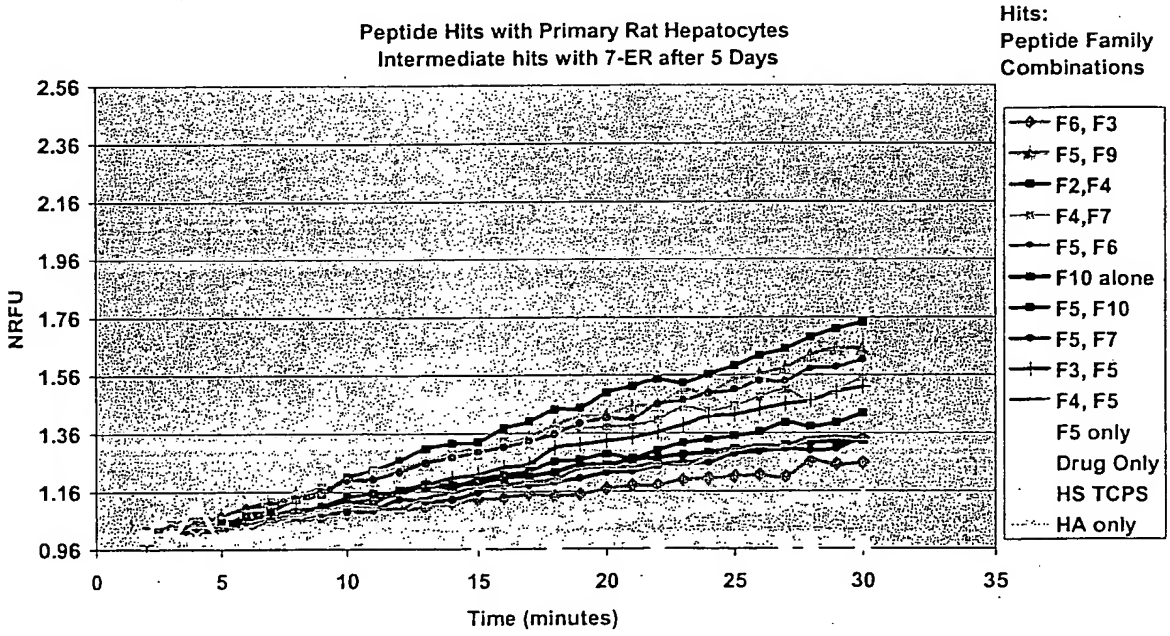


Figure 1B, intermediate CYP hits



Figures for IDR "Attachment and maintenance of function of primary hepatocytes" by RD Guarino et al. F- values refer to peptide families as defined in text of IDR

Figure 2A: albumin secretion of cells in wells with strong CYP hits

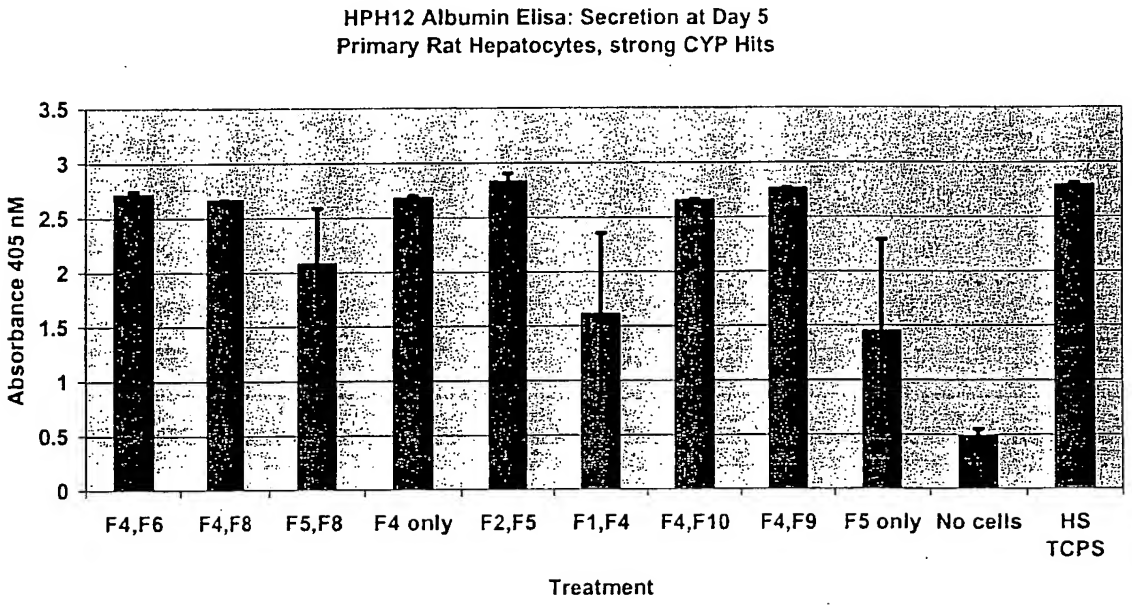
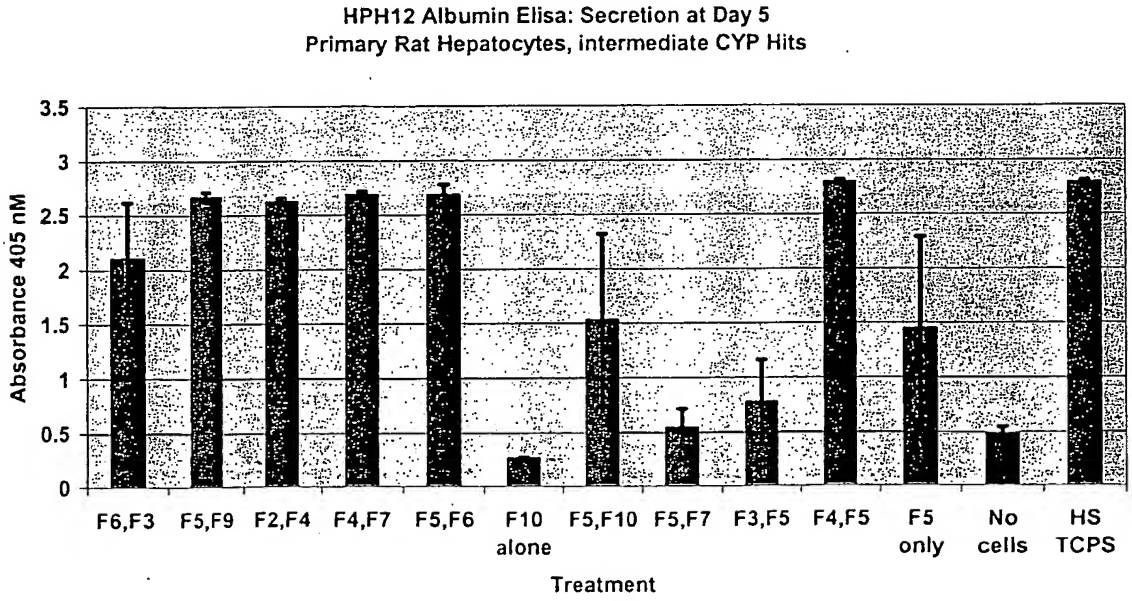


Figure 2B albumin secretion of cells in wells with intermediate CYP hits



Figures for IDR "Attachment and maintenance of function of primary hepatocytes" by RD Guarino et al, F- values refer to peptide families as defined in text of IDR

Figure 3 Peptide well hits for attachment of primary hepatocytes

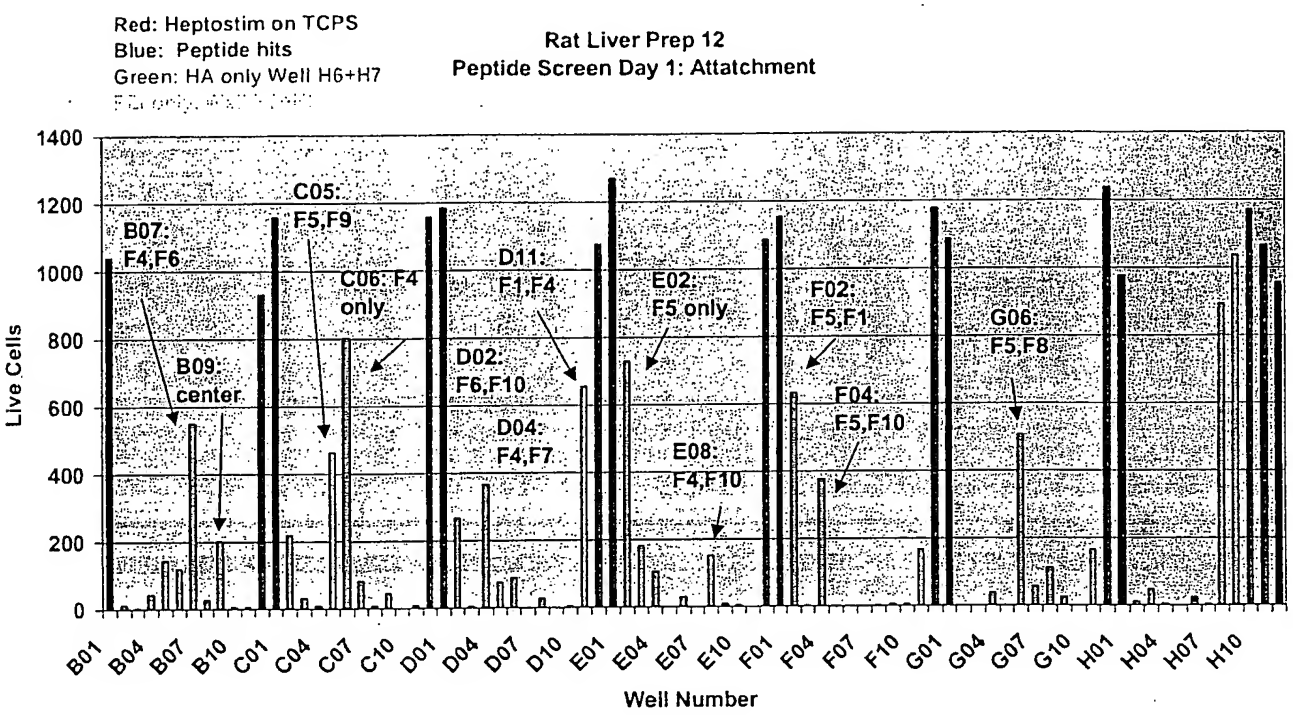


Figure 4 Well hits for peptide families survival of hepatocytes after 5 days in culture shows which families had surviving cells after 5 days using nuclear staining.